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OLUME XXVII

NUMBER 2

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

August, 1945

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

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BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

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This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

PERIPHERAL NEURITIS

Clinical and Physiological Observations on a Series of Twenty Cases of Unknown Etiology

MAJOR ABNER M. HARVEY,

Medical Corps, Army of the United States;

STEPHEN W. KUFFLER,

Research Fellow, Kanematsu Memorial Institute of Pathology, Sydney, Australia

AND

CAPTAIN JOHN B. TREDWAY,

Medical Corps, Army of the United States

INTRODUCTION

During the past few months twenty cases of peripheral neuritis have been studied in the 118th General Hospital in which the abnormality developed spontaneously and was confined for the most part to a single nerve. The common peroneal nerve was involved in sixteen, the axillary in three, and the radial nerve in one. All of the patients had recently been in the tropics and, in certain instances, the onset was associated with an attack of malarial fever. The cases were, for the most part, not observed until several weeks after the onset of their neuritis. In the majority there was no history of alcoholism, pharyngeal or dermal diphtheria were not present, the diet was adequate, and no cause for the development of the neuritis could be found. The onset was usually sudden with both motor and sensory involvement. The prognosis was good, and in most instances function returned within four to six months. The purpose of this communication is to present a clinical analysis of this group of cases, and to report a series of physiological observations on the nature of the changes in peripheral neuritis. The effect of quinine, atabrine and thiamin chloride on the course of the neuritis has been observed.

A. CLINICAL ANALYSIS OF THE CASES

1. *Age Distribution:* The patients varied in age from 22 to 48 years with the average approximately 30 years.

2. *Mode of Onset, Relation to Malaria and Environmental Factors:* In sixteen of the cases the onset of paralysis was sudden. Many awakened in the morning to find weakness of an extremity which had been perfectly normal the day before. In general, the weakness reached its greatest degree on the day of onset, but in a few instances the patient noted rapidly increasing weakness, which reached its maximum at the end of two or three days. The development of the weakness was "gradual" in only four of the patients with slow progression over a period of four to six weeks.

The onset of paralysis in eight of the cases was in association with an attack of benign tertian malaria. The time interval between the attack, and the appearance of the symptoms of neuritis was as follows:

Same day as clinical malarial attack	1 case
Three days after	1 case
Five days after	1 case
Seven days after	4 cases
Eight days after	1 case

Of the remaining twelve patients, ten gave no history of malaria and blood smears were negative for malarial parasites. Two patients did not have their episode of peripheral neuritis in any close temporal relationship to malarial fever, but had attacks of malaria before and after the onset of the peripheral neuritis.

There was no difference between those cases associated with malaria, and the others in regard to clinical features, severity or rate of recovery. All the patients had service in the tropics, the shortest period being one month and the longest eleven months. Of the twenty cases, four developed when the patient was out of the tropics; but in two of these the symptoms appeared one week after a malarial paroxysm. The other two patients had left the tropics only five and fourteen days prior to onset.

3. *Symptomatology:* All of the patients complained of weakness of an extremity. Five had a spontaneous type of pain during the course of the disease, which appeared early and lasted from a few days to two weeks after the onset of the weakness.

In the three cases of axillary nerve involvement pain was a conspicuous feature, being described as sharp or burning in character

and localized to the affected shoulder. In two cases of peroneal nerve palsy pain of a similar nature was located in the foot.

In nine sufficient sensory loss was present to cause a complaint of numbness. All of these had peroneal nerve palsies, and an area of numbness confined to the dorsum of the foot or lateral surface of the affected leg, which developed early in the course of the disease and persisted for a much longer period than the spontaneous pain.

Paresthesias were noted by only five patients, and described usually as a feeling of "pins and needles". They were never a very disturbing symptom, appeared early, and gradually disappeared over the course of a few days up to a few weeks.

4. *General Physical Findings:* General physical examination showed no unusual features, other than those associated with the nerve paralysis. Since some of the patients were seen when well on the road to recovery, the sensory and motor changes observed were probably less pronounced than at the onset.

5. *Motor and Sensory Phenomena:* (muscle function was graded according to the following criteria:

Power of Muscle (per cent):

0–10: Contraction may be felt but there is no apparent movement.
10–40: Muscle moves the part through partial arc of motion with gravity eliminated.
50: Completes whole arc of motion against gravity.
60– 90: Completes arc of motion against gravity plus variable amounts of resistance.
90–100: Completes arc of motion against gravity and maximum amount of resistance several times without signs of fatigue.

(a) The one case of radial neuritis, when examined approximately two weeks after onset showed 50 per cent function of the radial-innervated muscles of the forearm, and there were no objective sensory disturbances.

(b) The three cases of axillary nerve palsy had the most pronounced loss of motor function. Two of these cases were examined three and four months after onset, respectively, and the third developed in the hospital while the patient was under observation because of skin disease. All had complete loss of function in the deltoid muscle

accompanied by a moderate degree of atrophy. One patient had definite tenderness to pressure over the deltoid muscle.

Although the three patients are listed as having axillary nerve paralysis, two of them showed evidences of additional nerve involvement. One patient had some atrophy of the other shoulder girdle

FIG. 1. PERIPHERAL NEURITIS LOCALIZED TO THE SHOULDER GIRDLE AND INVOLVING MAINLY THE AXILLARY NERVE

Photograph taken five months after onset of the paralysis

muscles on the same side. He also exhibited slight winging of the scapula, marked weakness of biceps and triceps muscles (with absent biceps and triceps reflexes), and only 60 per cent function of the forearm and hand muscles of the same side (Fig. 1). Obviously, the neuritis was not confined to the axillary nerve alone but in varying degrees to the entire brachial plexus. The second case had, in addition

to the loss of function of the deltoid, some weakness of the flexors of the fingers of the left hand, and of flexion and extension of the left elbow. In both patients the nerve involvement developed directly after an attack of malarial fever.

Two of these cases showed hypaesthesia and hypalgesia in the area of the axillary nerve distribution. However, in the first patient mentioned above there was an area of anesthesia and analgesia measuring 15 x 16 cm. over the lateral surface of the upper arm and shoulder.

(c) The sixteen patients with peroneal nerve palsies were first examined an average of four and a half weeks after the onset of symptoms. The strength of the involved muscles varied between 0 and 70 per cent, but in only three patients was any noticeable muscular atrophy present. A few complained of pain on pressure over the involved muscles. Five cases gave evidence of more marked involvement of the superficial than of the deep branch of the peroneal nerve. Bilateral involvement was present in four cases, the superficial peroneal being chiefly involved in two, and the common peroneal in the others. In all four the strength of the peroneal-innervated muscles varied from 60 to 80 per cent of normal function.

The sensory findings showed a wide range of variation and in eight cases sensation was apparently normal at the time of first examination. In general, the greater the motor involvement the more marked was the loss of sensation. In one case there was complete anesthesia and analgesia over the peroneal nerve distribution with a surrounding zone of intense hyperesthesia. In three of the others, all of whom were examined early in the course of the disease, there was an extensive area of diminished sensation over the lateral surface of the leg and the dorsum of the foot. In the remaining cases only very small areas of impaired cutaneous sensation were present, and in two of them ill-defined zones of hyperesthesia were found.

6. *Vasomotor Changes:* Striking vasomotor changes were present in only a few patients, in whom the neuritis was severe. They showed a warm, dry extremity even with the body exposed in a cool room. In one patient with a peroneal palsy, the foot became swollen and cyanotic when in a dependent position. No detailed studies such as those described by Wilkins and Kolb (1941) were carried out.

7. *Routine Laboratory Studies:* The only abnormality discovered on examination of the blood was an eosinophilia in three cases with

values of 6 per cent, 23 per cent, and 24 per cent respectively. One case showing eosinophilia had hookworm ova in the stool, but in the other two no parasites were found. The spinal fluid was examined in seven cases, with normal results in all (Wassermann, cells, mastic curve, and protein). When indicated X-rays of the involved limb were taken and foci of infection searched for, but with negative results.

B. ELECTROMYOGRAPHIC STUDIES

Electromyograms in response to maximal stimulation of the motor nerve have been recorded in six cases with involvement of the peroneal nerve, and in one with involvement of the radial nerve. These studies provide an objective estimate of the remaining function and furnish evidence of the type of physiological change which accompanies lesions of the nerve fibers in peripheral neuritis. These abnormalities are distinctly different from the type of change described in diseases involving the region of the neuro-muscular junction (Harvey and Masland, 1941) and of the muscle fibers themselves (Gammon, Harvey and Masland, 1941). The records are made in response to a maximal nerve stimulus so that the results are comparable from day to day, and the progress of the lesion can be determined as well as the effect of various drugs on the state of the nerve function.

1. *Method:* The electromyograms were made according to the method described by Harvey and Masland (1941). Stimulation was by means of condenser discharges using gas discharge tube circuits, or through the secondary circuit of an induction coil. When two impulses were applied, the short interval of time between them was regulated by means of a Lucas pendulum.

Small silver electrodes were used for recording and the action potentials were led through a condenser-coupled amplifier to a cathode ray tube.

The radial nerve was stimulated over its course from the medial to the lateral side of the humerus. Records were made through the skin overlying the brachioradialis and extensor carpi radialis muscles.

The common peroneal nerve was stimulated behind the head of the fibula at the lateral side of the popliteal space. The muscle action potential was recorded from the tibialis anterior muscle ten to

fifteen centimeters below the patella or three or four centimeters further laterally over the extensor digitorum longus and peroneus longus muscles.

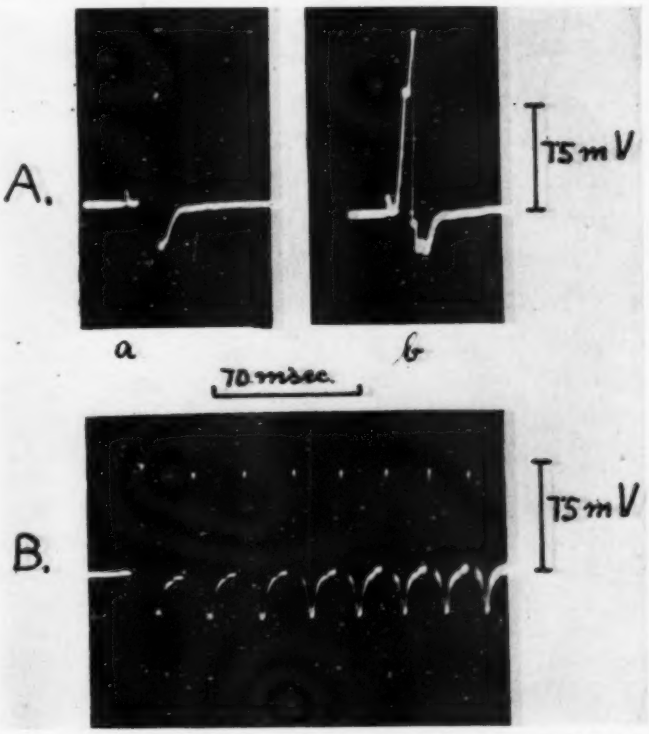

FIG. 2. NEURITIS OF PERONEAL NERVE, SIX WEEKS AFTER ONSET

Action potentials recorded from skin over tibialis anterior muscle in response to maximal stimulation of the common peroneal nerve.

A. a. Peripheral neuritis

b. Normal leg

B. Train of maximal nerve stimuli at 50 per second, neuritis leg.

2. *Results:* a. *Response to a Single Nerve Stimulus:* The voltage of the muscle action potential in response to a single maximal motor nerve stimulus was alway reduced in size, but was normal in shape and duration (Fig. 2A). This is very suggestive evidence that the

excitation wave spreads normally along the muscle fibers, and that there is no primary defect in their function as is present in such a disease as family periodic paralysis (Gammon, Harvey, and Masland, 1941). The reduction in voltage was proportional in degree to the

Peroneal Nerve Paralysis

	DAY OF ILLNESS	ACTION POTENTIAL		CLINICAL ESTIMATE OF FUNCTION
		mV	Per cent Normal	
Patient 1:	44	8.4	52	50
	49	8.4	52	
	56	8.1	50	
	63	8.7	54	
	70	11.4	71	
	77	11.4	71	
	85	12.7	79	
	95	12.4	78	
	99	12.0	75	80
Patient 2:	33	.95	8	0
	39	2.4	20	
	46	1.8	15	40
	53	5.1	42	40
	60	4.2	35	
	67	4.2	35	
	77	6.6	55	70
Patient 3:	19	5.6	46	60
Patient 4:	55	5.6	69	80
Patient 5:	21	10.3	79	80
Patient 6:	20	1.0	11	20

FIG. 3. Chart showing the function of the muscles in peripheral neuritis as estimated by electromyograms and by clinical testing. See text.

reduction in motor power, and correlated reasonably well with clinical estimates of the muscle strength made according to the method of Kendall and Kendall (1939). In Fig. 3 the voltage of the muscle action potential on the affected side is compared with that obtained from the normal side, and the length of time the observation was made after the onset of the illness is listed. It can be readily seen from this table that the severity of the involvement varied greatly.

In certain cases there was considerable variation in the degree of involvement of the branches of the peroneal nerve. For instance, in one examined on the 20th day following the onset of weakness, the potential recorded from the tibialis anterior muscle was 3.3 mV while that from the extensor digitorum longus was only 1 mV. Potentials recorded from the corresponding areas of the normal leg of the same individual were both 8.7 mV.

b. *Response to Two Nerve Stimuli at Short Intervals:* Two stimuli were applied to the nerve at intervals varying from a fraction of a millisecond up to fifty milliseconds (Fig. 4). The additional responses set up by the second nerve volley do not differ from those found in the normally functioning nerve of the opposite limb. This indicates that most of those nerve fibers which are affected in peripheral neuritis are completely inactive, while the others function normally. Otherwise, facilitation with the very close intervals, falling within the refractory period of the nerve would be expected. If a significant block in conduction at the neuro-muscular junction were present, facilitation should appear at the intervals outside of the refractory period of the nerve but within that of the muscle. In such a case additional muscle fibers would respond to the second stimulus due to the summation of end plate potentials set up by the two nerve volleys (Eccles, Katz, and Kuffler, 1941). Such neuromuscular facilitation has been described in partially curarized animals (Brown, 1938), and in certain stages of myasthenia gravis (Harvey, Lilienthal and Talbot, 1941).

c. *Effects of Tetanic Stimulation:* When a volley of motor nerve impulses was applied at the rate of fifty per second, the first eight responses had approximately the same voltage (Fig. 2B). In a few instances when the paralysis was very severe there was a gradual fall in voltage during the first six responses to about ninety per cent of the original value. This has not been consistent enough to be of any great significance. These observations are in striking contrast to the findings in cases of myasthenia gravis in which each successive volley at similar intervals produces a greatly reduced response.

d. *The Effect of Prostigmine:* Three patients were given 1.5 mgm. of prostigmine intramuscularly, and the responses to a single and to tetanic stimuli were studied before, and for a period of one hour after

its administration. In spite of the fact that spontaneous fasciculations developed in the normally innervated muscles no change in the response of the nerve under study could be detected. Likewise,

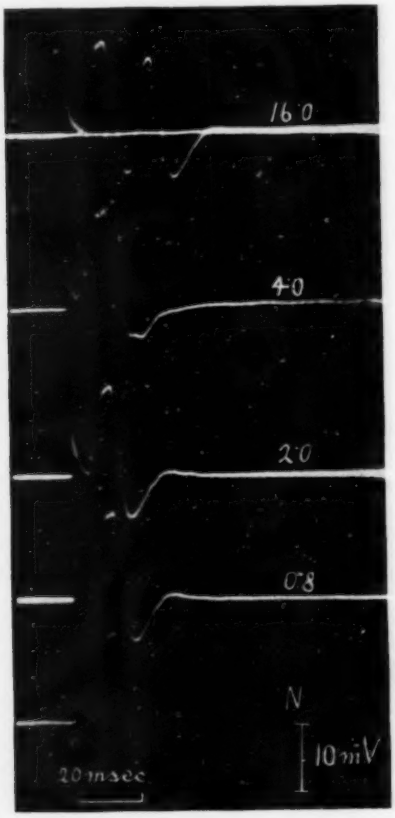

FIG. 4. NEURITIS OF PERONEAL NERVE, EIGHT WEEKS AFTER ONSET

Action potentials recorded from skin over extensor digitorum longus in response to maximal stimuli applied to common peroneal nerve. *N*, response following a single maximal nerve volley. *Other records*, two maximal nerve volleys (N1, N2) at intervals indicated in milliseconds. At 4.0 m.sec. N2 sets up a small additional response, while at 16.0 m.sec. most muscle fibers are activated for a second time. This series does not differ from the normal side except for the smaller size of the action potentials, which are about 50 per cent of normal voltage.

no difference was observed in the voluntary movements before and after the injection. These studies were done both at the height of the paralysis, and during the period when recovery was taking place and function improving. It was thought that during this latter period the function might be altered by prostigmine but such was not the case.

C. COURSE AND THERAPY

1. *Course:* Those patients who could be followed over a sufficiently long period of time showed, in general, a gradually progressive improvement in the strength of the affected muscles. When present, vasomotor and sensory changes tended to improve more rapidly than the motor weakness.

In contrast to the general tendency toward slow, progressive improvement, two of the cases of axillary nerve palsy failed to show any return in function after six months. In the third case the follow-up period was too short to expect any recovery.

The one case of radial nerve paralysis improved slowly over the course of two months to about eighty per cent of normal strength.

Nine of the fourteen cases of peroneal neuritis were followed over a long enough period of time to gain an accurate evaluation of their course. In all but one recovery was satisfactory. One case relapsed while under observation. He had a peroneal neuritis which developed in the absence of any clinical malaria, and gradually improved over the course of three months. At the end of that time there was a sudden increase in weakness, without any apparent precipitating factor. The patient could not be followed for any appreciable time after the relapse.

In most of these patients it was possible to obtain electromyograms at weekly intervals from a time shortly after the onset of the paralysis through most of the recovery period. Fig. 5 B, C, illustrate the recovery curve of two characteristic cases of peripheral neuritis. The degree of function is indicated by the voltage of the action potential in response to a single maximal motor nerve stimulus. Generally, there was a steady and progressive increase in function until the potential reached a value 70 to 90 per cent of that obtained from the corresponding muscle on the normal side. The curve then flattened out, and during the ensuing five to six weeks remained stationary.

In some of the patients there was an appreciable fluctuation in the action potential values during the period of functional return (Fig. 5C). This was also reflected clinically. The potential would show a variation of 1 or 2 mV from week to week, but with a tendency to climb toward normal. The period of progressive increase in the voltage of the action potential preceded by a short interval of time (seven to fourteen days) a demonstrable clinical improvement. When

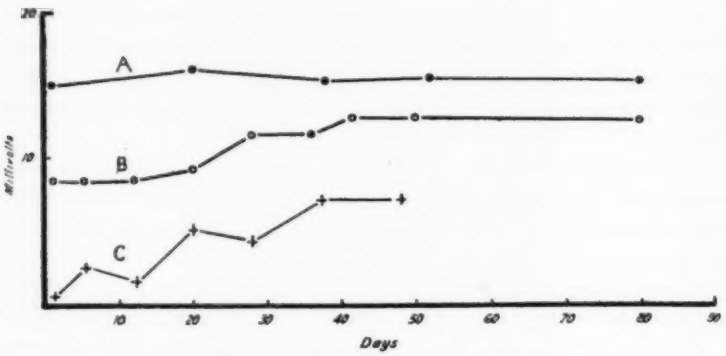

FIG. 5. PERIPHERAL NEURITIS, PERONEAL NERVE

Recovery curve obtained by plotting size of muscle action potentials (ordinates) against time (abscissae).

Case 1: First electromyogram obtained 44 days after onset of paralysis.
A. normal leg.
B. neuritis leg.

Case 2: First electromyogram obtained 33 days after onset of paralysis.
C. neuritis leg.

The normal leg showed a potential of 10 mV which is not plotted.

the maximum degree of functional return as evidenced by a comparison of action potential values, had been reached, estimates of degree of strength made independently by clinical means coincided very closely (Fig. 3). In none of the cases studied did the return of function reach 100 per cent as determined by a comparison of the action potential from corresponding muscles on the two sides. This is suggestive evidence that certain of the nerve fibers are permanently damaged and do not regenerate.

2. *Treatment:* The main object of the treatment of these patients has been rest in a well protected position to prevent stretching of the weak muscles. This has been carried out by the use of protective plaster shells to hold the limb in neutral position or by an adequate brace. When the muscle function was below 50 per cent exercises were given with assistance to the weak muscles using the test movements described by Kendall and Kendall (1939) as the basis for the localization of the exercises. Weak muscles were not allowed full range of motion so that constant protection was always maintained. The degree of exercise was carefully graded and increased gradually but never to the point of fatigue. Care was taken to prevent "substitution" movements during the exercise as this would result in relatively strong muscles being exercised at the expense of the weak ones. When the power was greater than 50 per cent, active exercises were begun provided the opposing group of muscles also had greater than 50 per cent power. Weak muscles were always placed in a neutral position before starting exercise movements. It was often necessary to apply heat and stretching exercises to opposing muscles e.g. to the tendo achilles when there was weakness of the dorsiflexors. The patient was taught to consciously relax the opposing muscles. Whenever weight-bearing muscles were involved the patient was taught correct balance, a general muscle check was done, abdominal muscles were built to normal strength, and proper shoe corrections applied if necessary. Prevention of fatigue was always stressed and the patient was encouraged to rest before the point of fatigue was reached. It was felt that electrical stimulation could not be sufficiently controlled, and it was not used as a form of treatment in any of these patients.

D. FACTORS HAVING A POSSIBLE INFLUENCE ON THE DEVELOPMENT OF THE PERIPHERAL NEURITIS

1. *Drugs:* All of the patients had been on anti-malarial suppressive treatment prior to the development of the peripheral neuritis. Significant data on the type and amount of suppressive therapy are gvien in table 1.

The amount of drug ingested in the above periods can only be roughly estimated because of individual variations in following the

recommended suppressive dosage (atabrine, 0.6 gm. per week and quinine 0.6 gm. per day).

Two patients with involvement of the peroneal nerve, in whom the function was 35 per cent in one and 52 per cent of normal in the other, were given 0.2 gm. of atabrine orally three times daily for a period of one week and 0.1 gm. of atabrine three times daily for the next four weeks. Repeated electromyographic studies were made during this period, but no deleterious effect of the drug could be detected. Clinically, the strength remained the same. The voltage of the action potential did not decrease, and the usual response to a

TABLE 1

SUPPRESSIVE DRUG	AVER. DURATION OF SUPPRESSIVE TREATMENT PRIOR TO ONSET OF NEURITIS	NO. OF CASES	TYPE OF PERIPHERAL NEURITIS
Quinine only	about six weeks	three	2 axillary 1 peroneal
Atabrine only	about 24 weeks	seven	5 peroneal 1 axillary 1 radial
Atabrine and quinine	about 20 weeks	five	5 peroneal
Atabrine only	not known	two	2 peroneal
Atabrine and quinine	not known	one	1 peroneal

train of nerve stimuli showed no variation. Similar studies were done in two cases during a period of one week when the patients were receiving 0.6 gm. of quinine three times a day by mouth. Again there was no increase in the weakness or decrease in the voltage of the muscle action potentials.

2. *Diet:* In the consideration of what part a deficient vitamin intake might play in the development of the peripheral neuritis, each patient was questioned in some detail concerning his diet while in the tropics and prior to the onset of the neuritis. In only two cases could the patient's diet be called inadequate, from the standpoint of vitamin intake, and each had been on the diet for a period of about two months.

The diet of the remaining cases was classed as "adequate" to "good", and it is worthy of note that one patient who developed a severe peroneal neuritis had taken polyvitamin capsules regularly.

Two of the patients were placed for a period of one month on a diet which consisted of no foods containing significant amounts of thiamin. The function of the nerve was less than fifty per cent of normal in each instance and had been stationary for a period of several weeks. During the course of the observations the function, as estimated clinically and by recording of the muscle action potentials, remained essentially unchanged while the patient was on this diet which was low in B complex. At the end of one month thiamin chloride (25 mgm) was administered intravenously; followed by doses of 10 mgm three times a day for one month. There was no immediate change in nerve function as indicated by study of the muscle action potentials in response to standard nerve stimuli, and the recovery curve did not appear to be appreciably accelerated.

3. *Complicating Diseases:* The relation of some of the cases to malarial fever has already been pointed out. Six patients had an attack of malaria following the onset of their peripheral neuritis, and in no instance was any exacerbation of the neuritis noted. This observation has some bearing on the possible character of this type of peripheral nerve lesion. In the cases of neuritis characteristic of malarial fever (Harvey, 1944, unpublished observations) the symptoms and objective findings were almost uniformly accentuated during relapses of the malaria. Not any of the patients had had typhus fever or diphtheria, one had a phlebitis of the involved leg, one had a non-specific dermatitis of the hands and feet, and one had a lichen planus-like skin lesion.

DISCUSSION

A series of cases of peripheral neuritis has been described, in the majority of which the peroneal nerve was involved. When this nerve was affected the degree of recovery was uniformly satisfactory. The cause of these neuritides was not certain, and the only feature which was common to all of them was a period of duty in the tropics. In addition, eight of the cases had their onset in relationship to an attack of benign tertian malarial fever. In the majority of cases

the onset was sudden, and the motor weakness and sensory involvement reached a maximum within one to two days of the time of onset.

Peripheral neuritis has been frequently reported as a complication of malarial fever (cf. Anderson). In the cases described by Conti (1910), the neuritis usually began suddenly and tended to produce paralysis with intense pain, but in other instances the onset was more gradual with paresthesias and lightning pains which were followed by slowly increasing weakness of the involved region. In almost all of these cases the descriptions are not given in sufficient detail to clarify the clinical course of the neurological changes in relation to the acute attack of malaria. In the large majority infection was due to the malignant tertian parasite. Many of the cases had a polyneuritis involving mainly the lower extremities.

During the same period in which the cases in the present series were studied, twelve cases of polyneuritis were also under observation, the majority of which showed a high spinal fluid protein but no increase in cell count. In all but two of these patients the onset followed an attack of benign tertian malarial fever, and all of the patients had been on duty in a tropical climate within a short time of the appearance of the symptoms. Spinal fluid examinations in the present series of cases with involvement of but a single peripheral nerve, however, showed no increase in protein content. During the same period of time a distinctive type of neuritis which appears to be characteristic of malarial fever was also observed in this hospital. Pain, hyperalgesia, increased sweating and heightened muscle tone were prominent features in these cases; and in contrast to the patients with peripheral neuritis reported in the present study, there was a pronounced increase in the symptoms with subsequent attacks of malarial fever. It is felt that the cases in the present series have no specific relationship to malarial fever, but that this disease together with other conditions brought about by tropical duty are merely precipitating factors. The evidence which has been described in several of the cases makes it extremely unlikely that either atabrine or quinine could have had an etiological rôle.

There have been recent reports of cases of localized neuritis which seem similar in most respects to those described in this communication. Spillane (1943) observed a series of forty-six patients with a localized neuritis in which an unusual degree of wasting of certain muscles

about the shoulder girdle of fairly rapid onset was the characteristic clinical picture. In seventeen the involvement was largely confined to the deltoid muscle with loss of sensation over the cutaneous zone of the circumflex nerve. In three instances the onset followed benign tertian malaria. The distribution of the paralysis, as in the cases of the present series, took a peripheral rather than a segmental character and for the most part one nerve was mainly affected. The author had also seen seven examples of unexplained paralysis of the external popliteal nerve and seven of radial nerve palsy. In addition, he quoted a personal communication from Macpherson and Clark (1943) stating that they had observed ten cases of isolated paralysis of the external popliteal nerve in soldiers serving in Malta. The onset was sudden, painless, and not accompanied by any signs of general infection. There was excellent recovery in most instances but a tendency to relapse was present in a few patients. In 1941, Wyburn-Mason drew attention to an increase in the number of patients suffering from brachial neuritis observed at a London Hospital. He examined forty-two cases between December, 1940 and August, 1941, and also pointed out that in a period of three months he had seen seven cases of isolated paralysis of the external popliteal nerve. Foot-drop and the characteristic sensory change were recorded. In one patient there was a simultaneous bout of brachial neuritis. Spillane stated that civilian practitioners whom he consulted in the Middle East had not seen such cases before, and that in his capacity as consultant neurologist there in 1914–18 Sir Purves-Stewart had not observed any. It appears, therefore, that there is a type of peripheral neuritis with involvement of the shoulder girdle and of the muscles innervated by the peroneal nerve which is appearing not only among troops in various parts of the world but also in the civilian population. There are as yet no facts available which give any definite information as to the etiology of this disease.

The studies on the physiological changes have yielded information of interest. However, many more observations must be made as the opportunity is presented, and by use of the simplified methods of studying nerve-muscle function now available further data can be gathered on the course of this disease and the influence of various forms of treatment.

The present observations show that during those stages when the

investigations were made, there was no abnormality of the neuro-muscular conduction or of the spread of the excitation wave along the muscle fiber. The results could best be interpreted by assuming that certain nerve fibers had ceased to function while activity in the remaining ones was normal. This was supported by the fact that no alteration in the level of function of the nerve as a whole could be brought about by various forms of activity or by the action of drugs such as prostigmine. However, a neuro-muscular component may yet be detected during the early stages of the neuritis when the paralysis is developing. From the curve of recovery it seems reasonable to assume that the regeneration of the fibers must be complete before any improvement in function of the nerve becomes evident.

A simple program of treatment has been employed. Of most importance were the provision of a suitable support for the weak muscles and careful instruction of the patient in order to prevent further damage by improper use of the involved limb. When the patients were first observed a careful check of muscle function was done and the results of this were used as a basis for planning treatment. This was of particular importance when weight bearing muscles were involved as faulty body mechanics during the period of weakness could leave other serious defects in posture. Carefully graded exercises were instituted and the amount of exercise and its character was determined by the degree of functional loss. Treatment was given to individual muscle groups in the same manner that the muscle testing was done (Kendall and Kendall). It was not felt that electrical stimulation could be sufficiently localized or controlled to employ it as a method of treatment.

SUMMARY

1. Twenty cases of peripheral neuritis have been observed in which all of the patients had been on tropical duty and in eight of which the onset was related to an attack of malarial fever. The common peroneal nerve was involved in sixteen, the axillary nerve in three, and the radial nerve in one.

2. Electromyograms were made in response to maximal stimulation of the motor nerve in six cases with involvement of the peroneal nerve.

a. The loss of function indicated by the reduced voltage of the muscle action potential is a result of lesions of the nerve fibers only while neuro-muscular transmission and muscle conduction is normal.
b. The recovery curve was followed in several cases by recording the action potentials at frequent intervals.
c. Atabrine and quinine had no influence on the degree or rapidity of the recovery in these patients.

3. In all cases with peroneal nerve paralysis the prognosis was good. Treatment consisted of proper support of the involved muscles, graded exercises under supervision of trained physiotherapists, and a balanced diet supplemented by vitamins of the B complex.

All of the muscle checks described and the physiotherapy treatments were supervised by Lelia Griffin, 1st Lt., Physiotherapist, Army of the United States.

ILLUSTRATIVE CASE REPORTS

1. *Paralysis of the Peroneal Nerve:* This 36 year old Medical Officer went to a tropical island on October 1, 1943. He had a mild attack of diarrhoea shortly after his arrival, but there were no other illnesses. He took atabrine irregularly. On December 17, 1943 upon awakening he found that he had a foot-drop, and no power of eversion in his right foot. There was some loss of sensation over the dorsum of the foot and along the lateral aspect of the lower leg with a zone of hyperesthesia around it. The weakness became more pronounced during the day and had reached its maximum degree by the following morning. On pressure over the peroneal nerve there was no tenderness but reference of pain just along the hyperesthetic areas was produced. Five days after the onset tenderness appeared on pressure over the nerve which felt as though it was swollen. He received heat to the leg, but pain was so intense following the treatments that they were discontinued. Whenever the leg was dependent a burning pain developed and the foot became cyanotic and puffy in appearance.

Examination at the 118th General Hospital on January 8, 1944 revealed $\frac{3}{4}$ inch atrophy of the calf. There was marked reduction in power of the toe extensors, particularly the extensor hallucis. The

evertors of the foot were functionless, and strength in the tibialis anterior was less than fifty per cent. There was extensive loss of cutaneous sensation over the dorsum of the foot and the lateral aspect of the lower leg. This area was surrounded by a zone of fairly intense hyperesthesia and hyperalgesia. The foot was slightly swollen and felt warm and dry.

The patient was placed on a diet high in vitamins. A brace was fitted for wear during the day, and at night a posterior shell was applied. He was given graded exercises as outlined above, but no electrical stimulation was applied. Recovery was complete five months after the onset of the illness.

2. *Localized Neuritis about the Shoulder Girdle involving mainly the Axillary Nerve:* This patient was a 24 year old private who went to a tropical island on September 20, 1942. November 12, 1942 he had a febrile illness which was relieved by quinine. November 30, 1942 he was wounded in the right buttock by a sniper's bullet and while in the hospital on December 6, 1942 had an attack of malarial fever due to P. vivax for which he was treated with quinine, atabrine and plasmochin. On December 11, 1942 severe, sharp, throbbing pain developed in the right upper arm, and he gradually lost the power to abduct the arm. The pain continued for about three weeks, and then became milder and intermittent. December 26, 1942 he had a relapse of his malarial fever which caused no increase in the symptoms referable to his arm. On January 6, 1943 he was examined at a General Hospital and found to be unable to abduct the right arm, but no conspicuous atrophy was noted. The most marked weakness was found to be in the deltoid and the supra- and infra-spinatus muscles. There was anesthesia in the cutaneous zone of the axillary nerve. X-rays of the cervical spine and shoulder were normal. The blood serological test for syphilis was negative, and examination of the spinal fluid revealed no abnormalities. When examined on March 27, 1943 there was marked atrophy of the muscles about the right shoulder, particularly the deltoid. There was complete loss of abduction and internal and external rotation of the shoulder were much below normal strength.

June 1, 1943 he was admitted to the 118th General Hospital. At that time there was marked atrophy about the shoulder and in the

infra- and supra-scapular regions. (See Fig. 1.) All of the muscles in these areas functioned poorly, and the deltoid muscle did not respond at all. There was slight winging of the scapula. The triceps and biceps muscles were very weak, and the corresponding tendon reflexes almost unobtainable. There had been no return of function when the patient was re-examined in August, 1943—nine months after the onset of the neuritis.

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TOXICITY OF SULFANILAMIDE ON HIGHER NERVOUS ACTIVITY

W. HORSLEY GANTT AND E. K. MARSHALL, JR.

Pavlovian Laboratory of Phipps Clinic and Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University

INTRODUCTION

The observation of one of us (E.K.M.) that temporary blindness has followed large doses of sulfanilamide suggested the use of the cr[1] method to determine the cortical effect of this drug. Toxic symptoms have been described[2] (1) from doses as small as 0.5 gms. per kgm. in dogs. Marshall et al. (v. supra) state that 0.1 to 0.2 gms. per kgm. have been given to many dogs without observable symptoms. They state that 1 gm. per kgm. by mouth in 7 dogs gave no symptoms in one, slight ataxia in another and in 5 "salivation, vomiting, diarrhea, hyperpnea, excitement, muscular weakness, ataxia, signs of stimulation and depression of the central nervous system leading to a condition of spastic rigidity of the limbs and hypesthesia. The early stages of the poisoning resemble those seen in dogs given large doses of ethyl alcohol; the later stages in many respects are similar to those shown by a decorticated dog. The placing reactions, known to be due to the cerebral cortex, were absent in dogs after these large doses of sulfanilamide, and the dogs appeared to be blind. All symptoms disappeared in from ten to twelve hours, and the animals appeared normal".

In various experiments the conditional reflex method has been shown to be one of the most delicate measures of a disturbance of cortical equilibrium in the action of drugs and other toxins in both dogs and human beings. The conditional reflex is frequently the earliest indicator of a disturbance of cortical function; a deviation from the normal conditional reflexes is seen long before neurological

[1] Conditional (conditioned) reflex abbreviated cr; unconditional (-ed) reflex abbreviated UR.

[2] E. K. Marshall, W. C. Cutting and K. Emerson (Toxicity of Sulfanilamide, J. A. M. A., **110:** 252, 1938).

changes. Thus a deficiency of only three or four days of vitamin B complex results in the loss of differentiation of the crs although there is no corresponding change in the observable behavior, and subjection to an altitude of 16,000 feet in old dogs causes a marked impairment of cr function without neurological changes (2).

METHOD

Four dogs, aged 2 to 12 years respectively who had been studied in the laboratory for 2 to 8 years, were given orally 0.1, 0.25, 0.50 gms. sulfanilamide per kgm. body weight. Two of the dogs had salivary crs to food, and two of them had motor cerebellar crs.

In the two dogs with salivary crs, on test days the sulfanilamide was given about 9 a.m. and the crs measured every few minutes for the next 4 to 7 hours and again on the following day to ascertain the after effect. Cr readings for a period of 30 seconds made at 30 to 60 minute intervals were used in compiling our data as they provide more uniform results than the 10 second records. The animal was taken out for a rest period once or twice during the day.

Blood was withdrawn from a foreleg vein, $\frac{1}{2}$ hour after administration of sulfanilamide and thereafter every hour to obtain the blood concentration.

As the dog is not usually confined in the camera for more than one or two hours, controls were run to ascertain the effect of the prolongation of the work period as well as the numerous repetitions of the conditional stimuli on the size of the crs.

The effect of the sulfanilamide was measured on both the URs and crs from visual and auditory stimuli. The visual stimulus was a flashing 60 watt lamp about a foot distant from the dog: it could be considered a strong visual stimulus but not as strong as the strong auditory because visual stimuli in dogs are generally weaker than auditory. The light was reenforced by 8 gm. food. The auditory stimuli were two: the bubbling sound, reenforced by a large amount of food (9 gm.) and therefore strong, and the metronome, weak because it was reenforced by a small amount of food (1.5 gm.). As the size of the cr is determined by the amount of food, as well as by the type and intensity of the conditional stimulus, there was thus both a strong and a weak auditory cr and a strong visual cr (2).

RESULTS

Sechs, age about 6 years, had been observed in the laboratory since 1934; he died in 1944 after anoxia and metrazol convulsions. He had well established crs to a visul stimulus (light flashing forty times a minute) and to the two auditory stimuli. The flashing light and the bubbling sound were reenforced with 12 gms. of food and the metronome with 2 gms. of food.

Sechs was given sulfanilamide 22 Dec., 1937, 4 Jan., 8 Feb., 23 Feb., 16 Mar., 19 Oct., 31 Oct., 28 Nov., 7 Dec., 1938.

After the small doses (.1–.2 gms. per kgm.) of sulfanilamide in Sechs there was no observable change in the behavior, but .5 gms. per kgm. produced some ataxia—incoordination in trying to get the food and falling when he stepped over the door sill of the camera, though he was able to jump on the table.

Billy was brought into the laboratory about 1931. He was a stable active animal whose cr readings had been regular. In 1932 he was used in alcohol and caffein experiments (3), and later in routine measurements of the crs, from 1932-1937, when the first sulfanilamide experiments were done. He died in 1941, age about 14.

Sulfanilamide was given to Billy on 22 Dec., 1937, 14 Jan., 16 Feb., 21 Dec., 1938, and 9 Jan., 1939. Controls were run on the intervening days. Like Sechs he showed no effects with the small doses. After 9½ gms. (0.5 gm. per kgm.) he was unsteady on his feet, somewhat incoordinated in opening the lid of the food box—he required about 2 seconds for this movement which he usually made instantaneously—the length of time it took him to eat the food was prolonged from a normal of 25 seconds to 45 seconds. With this large dose Billy appeared almost like a dog who had had 3 cc. alcohol per kgm. In running around the room he bumped into things but did not fall, though sometimes when attempting to take food, he had trouble finding it, fell down and cut his mouth. Often Billy was unable to jump onto the stand after sulfanilamide. He fell over when standing on three legs to urinate. Six hours after administration of the sulfanilamide the ataxia was much diminished.

In both Billy and Sechs the following changes occurred: the placing reflexes were normal, also the corneal reflex was present, though the wink (conditioned) reflex was absent. The pupils reacted to light.

TABLE 1

Effect of Sulfanilamide on Conditional Reflexes

DATE AND DURATION OF TESTS	DOSE PER KGM. BODY WT.	AMT & TIME MAXIMUM BLOOD CONCENTRATION	MAXIMUM ACTION ON CRS & CORRESPONDING BLOOD CONCENTRATION	EFFECT ON CONDITIONAL REFLEXES			
				visual		auditory	
				normal (before sulf.)	after sulf.	normal	after sulf.
Sechs							
1938 8 Feb. 7 hrs.	0.50 gm.	45 mgm. 5 hrs.	35 mgm. 3–8 hrs.	130	0	strong cr 90 | 210 weak cr 80 | 0	
23 Feb. 7 hrs.	0.25 gm.	25 mgm. 2–4 hrs.	25 mgm. 2 hrs.	80	30	—	—
16 Mar. 6 hrs.	0.50 gm. divided dosage	30 mgm. 2½–7 hrs.	30 mgm. 5 hrs.	280		strong cr 190 | 290	
19 Oct. 6 hrs.	0.25 gm.	18 mgm. 2–4½ hrs.	14 mgm. 4½ hrs.	60	0	strong cr 90 | 170	
31 Oct. 3 hrs.	0.25 gm.	22 mgm. 1–4 hrs.	22 mgm. 3 hrs.	180	80	strong cr 195 | 205	
28 Nov. 7 hrs.	0.50	51 mgm. 1–3 hrs.	40 mgm. 2–3 hrs.	160	40	strong cr 250 | 120	
7 Dec. 6 hrs.	0.50	51 mgm. ¾–3 hrs.	49 mgm. ¾–3 hrs.	240	0		
Average....	0.38 gm.	34 mgm. 2–4½ hrs.	31 mgm. 3–4 hrs.	160	33	150	165
Billy							
14 Jan.	0.50	36 mgm. 5 hrs.	20 mgm. 1 hr.	280	0	strong cr 600 | 300	
16 Feb.	0.50	38 mgm. 3 hrs.	35 mgm. 2 hrs.	280	0	strong cr 620 | 300 weak cr 80 | 120	

TABLE 1—*Concluded*

DATE AND DURATION OF TESTS	DOSE PER KGM. BODY WT.	AMT & TIME MAXIMUM BLOOD CONCENTRATION	MAXIMUM ACTION ON CRS & CORRESPONDING BLOOD CONCENTRATION	EFFECT ON CONDITIONAL REFLEXES			
				visual		auditory	
				normal (before sulf.)	after sulf.	normal	after sulf.
Billy							
21 Dec. 6 hrs.	0.38	58 mgm. 1–3 hrs.	58 mgm. 1–3 hrs.	120	40	weak cr 300 | 80	
9 Jan. '39 6 hrs.	0.38	49 mgm. 2–4 hrs.	49 mgm. 2–6 hrs.	240	10	weak cr 410 | 70	
Average....	0.43 gm.	43 mgm. 2.6–3.6 hrs.	40 mgm. 1½–3 hrs.	216	10	strong cr 610 | 300 weak cr 270 | 90	

The orienting reflex, viz., the turning of the animal to a new stimulus, became much increased; the appearance of this subcortical reflex is an evidence of inhibition of cortical function. The motor component of the crs was markedly diminished after the large dose of sulfanilamide.

The results of the experiments with the salivary crs are tabulated above. (See table 1.) The blood sulfanilamide reached a maximum of 51 mgm. percent in one hour in Sechs and 60 mgm. percent in one hour in Billy. Only a slight drop occurred during the five or six hour duration of the experiment.

The UR secretion of saliva was slightly decreased by the sulfanilamide.

The visual stimuli were markedly decreased in intensity by the sulfanilamide. The intensity of the weak auditory stimulus was also diminished but less so than the somewhat stronger visual stimulus. The strong auditory stimulus was slightly changed. The maximum effect of the sulfanilamide was reached after one to two hours in Billy and after two or more hours in Sechs. Averages of the results in the seven experiments in Sechs and the four experiments in Billy, showed that the visual stimulus lost 77 percent of its

normal value and that the large auditory stimulus gained 90 percent in Sechs, and in Billy the visual stimulus lost 95 percent of its value, the weak auditory stimulus lost 72 percent and the large auditory 50 percent of its normal value. On the following day the visual and auditory stimuli were back to their normal values (figs. 1, and 2).

The general behavior as well as the crs were normal on the day following sulfanilamide and the sexual reflexes, tested in Sechs and

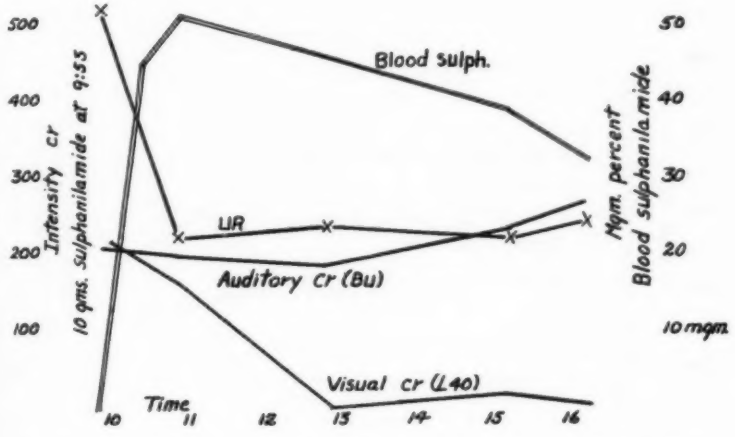

FIG. 1. SECHS, 7 DEC. 1938. 0.5 GM./KGM. BU = 3 BIS; L 40 = 2 BIS

Billy after the series were completed, showed no after effects of the drug.

Both of the dogs, observed for several years after these experiments, showed no permanent loss of cr function.

The effect of sulfanilamide on two dogs with motor crs elaborated to direct stimulation of the cerebellum, (4), by implanted electrodes, in which the conditioned and unconditioned reflexes were movements of the ears, neck muscles or foreleg without general struggling or pain reactions—was essentially the same as on the conditional food reflexes. Five gms. (0.5 gms. per kgm.) was given to dog no. 200 at 10:00 a.m.; before the sulfanilamide he gave a differentiation be-

tween a tone of 1000 cycles and a bell, giving the conditioned cerebellar movement to the tone but not to the bell. Six hours after the sulfanilamide the animal was ataxic and the positive cr to the tone had completely disappeared although the UR was present. On the next day the normal differentiation between tone and bell was present.

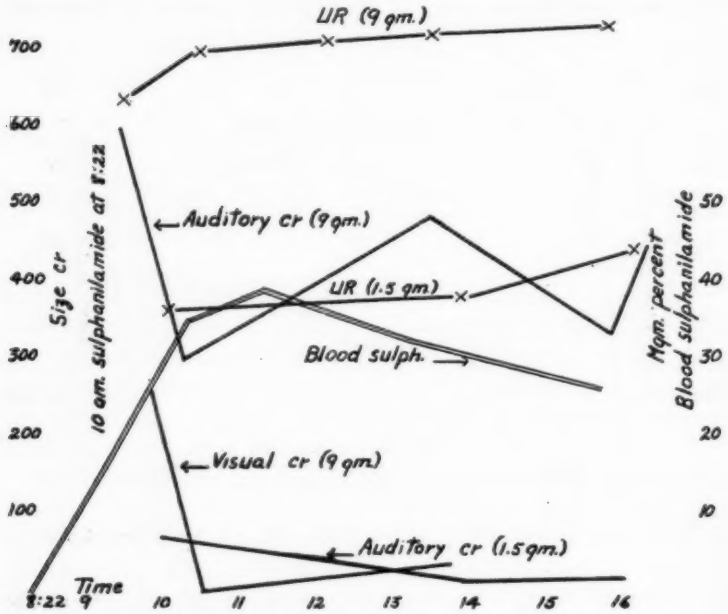

FIG. 2. BILLY. EFFECT SULFANILAMIDE. 0.5 GM./KGM.

Dog 201 was one who had normally a poor differentiation between the bell and tone. When given 0.5 gm. sulfanilamide on 28 Feb. there was no marked change in the cerebellar crs, but when 4 hours later an additional 0.7 gms. per kgm. was given, he became ataxic, vomited, completely lost the differentiation, failed to react to the positive crs, or showed a reversal of effect (i.e., the negative crs became larger than the positive) and even failed to give the UR, as shown in table 3.

TABLE 2

Control. Date and Duration Tests. Effect on Conditional Reflexes of Prolonged Experiment

	VISUAL		AUDITORY	
	beginning	end	beginning	end
Sechs				
28 Mar. 1939 7 hours	140	40	180 40	170 130
26 Jan. 1939 5 hours	25	125	100	180
Average.....	83	83	160	235
Bi'ıy				
18 Oct. 1938 5½ hours	120	220	260	140
12 Feb. 1935 9 hours			strong cr 400	405
			weak cr 100	110

TABLE 3

Effect of Sulfanilamide on Cerebellar Motor crs.

DATE	+ CS TONE	− CS BELL	UR
Control			
	percent	*percent*	
21 Feb. 1939.....	100	30	100%
23 Feb. 1939.....	100	70	100
24 Feb. 1939.....	100	30	100
Sulfanilamide			
28 Feb. 10:00, 0.5 gm. per kgm.			
10:40–11:40.....	80	20	100%
12:40.....	100	4	100
13:43.....	80	40	100
14:40, 0.7 gm. per kgm.			
15:50.....	0	20	No UR, ataxic, vomited at 16:15
17:06.....	20	20	No UR, less ataxic, but can't stand on hind legs

DISCUSSION

Controls were performed to determine the effect of prolonging the usual one hour experiment to five or more hours. We have previously shown that prolongation of the experiment up to as much as ten or more hours has little effect on the crs unless the animal is fed to satiation (5). Controls done on Sechs in 1938 and on Billy

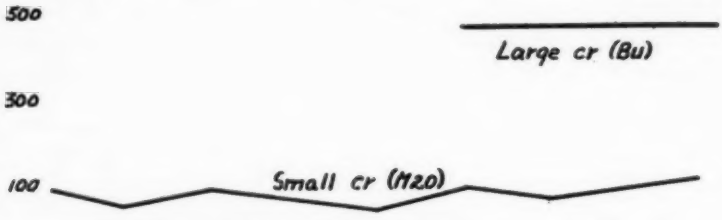

FIG. 3. BILLY, FEB. 1935. EFFECT REPETITION. 10 HRS.

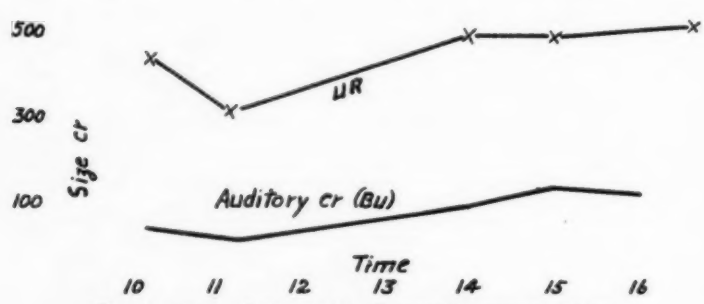

FIG. 4. SECHS, 17 OCT. 1938. EFFECT REPETITION 7 HRS.

in 1935 for the same number of hours as the sulfanilamide experiments show that there is no decrease in crs (figs. 3 and 4). Whenever the crs drop owing to repetition, the decrease is gradual and does not reach its minimum until the latter part of the experiment, whereas with sulfanilamide the decrease came rapidly after the administration of the sulfanilamide, usually within one or two hours, and sometimes rose toward the end of the experiment. In the controls for the purpose of determining the effect of the prolongation of the experiment there was no control on the influence of the venapuncture. It is

known that many such procedures may have an inhibiting action on the crs. This is true especially if the procedure involves conflict, but usually moderate pain per se does not have any but a transient effect on crs. Moreover the experiments with the small doses of sulfanilamide showed a less marked decrease of the crs and as blood was withdrawn in these they served as a control for the venapuncture, though it is still possible that this element of the procedure may have had a slight effect.

The effect of sulfanilamide on crs is, in a sense, opposite to that of alcohol (6). The latter drug, while having no true stimulating effect, produces apparent stimulation by releasing inhibition and reversing the rôle of the weak and the strong crs, but sulfanilamide exerts its depressing influence chiefly on the weak crs rather than on the strong. Although the weak crs, both visual and auditory, may be completely abolished by sulfanilamide, the normal relations between the strong and the weak are preserved, contrary to the action of alcohol.

The greatest effect of sulfanilamide is exerted upon visual crs, but as there is also an effect upon the weak auditory crs, the sulfanilamide may act non-specifically upon weak crs as well as upon the visual crs.

The effect upon unconditional salivary reflexes is that of moderate depression. As ataxia and incoordination was present in all the experiments where the crs were disturbed, sulfanilamide would appear to have a widespread cerebral influence, as well as involvement of both the cr and UR originating in the cerebellum. The disturbance in cr activity unlike that of alcohol does not appear until there are other motor effects.

The effect of sulfanilamide roughly parallels the blood sulfanilamide, though the maximum change in the crs may come earlier than the maximum blood concentration, and the crs may return to normal while the blood sulfanilamide is still high (figs. 1 and 2). The relationship between the total amount of sulfanilamide given and the depression of the crs varied somewhat with the state of the animal; the 5 gram doses in Sechs and the $7\frac{1}{2}$ gram in Billy often had as much influence as the 10 gram doses.

Although one of these dogs (Billy) was the old animal—dying of senility 2 years after the sulfanilamide experiments—the other 3 dogs were normal healthy animals.

A difference between the action in the dog and the human is possible as with some other drugs, although this difference judging by clinical reports is probably one of degree. In the frog for example, Rose (7) found the sulfonamides produced no ataxia, but then the frog has a very poorly developed cerebellum.

The amount of sulfanilamide required to produce impairment of the higher nervous activity in dogs was larger than the usual therapeutic doses, and no action could be detected in therapeutic dosage. Overdosing in humans produces ataxia and mental confusion roughly similar to what was seen in the dogs. Reed[3] reports in a 3 year old child after taking approximately 6 gm. sulfanilamide, convulsions, loss of consciousness and then weeping.

SUMMARY

Two dogs with previously well-established salivary conditional reflexes and two other dogs with cerebellar motor conditional reflexes were given sulfanilamide per ora in doses ranging from 0.1 to 0.5 gms. per kgm. body weight, and two other dogs with cerebellar conditional reflexes were given from 0.5 to 1.2 gms. per kgm. body weight. The external behavior of the animals showed no change with the small doses, moderate ataxia with the 0.2 gms. per kgm. and marked ataxia and incoordination with 0.5 gms. per kgm., the dog appearing as if he had had 2 to 3 cc. alcohol per kgm. Sulfanilamide in the large doses abolished the weak conditional reflexes and caused a decrease in both auditory and visual conditional reflexes, having the more pronounced action upon the visual conditional reflexes. The large dose of sulfanilamide abolished cerebellar conditional reflexes to auditory stimuli. Unlike alcohol, sulfanilamide does not change the relations between the values of excitatory and inhibitory conditional reflexes, but it has a generally depressing action upon all conditional reflexes. By the next day the conditional reflexes abolished by sulfanilamide had returned to normal, and there was no permanent impairment of cortical function with the doses used. The maximum effect of sulfanilamide upon the conditional reflexes is reached generally within an hour of administration, coinciding roughly with the maximum blood concentration of sulfanilamide, though

[3] Bull. M. J., Oct. 1, 1944, p. 535.

the conditional reflexes may be partially or wholly restored while the blood concentration is still high; the state of the animal is a factor as well as the drug in the size of the conditional reflex. It is important to note that sulfanilamide, one of the most toxic of the sulfonamides does not impair cortical reflexes until the therapeutic dose has been considerably exceeded.

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THE EFFECTS OF CRUDE AND PURIFIED PENICILLIN ON CONTINUOUS CULTURES OF NORMAL AND MALIGNANT CELLS[1]

G. O. GEY, M. K. GEY, F. INUI AND H. VEDDER

From the Division of Cellular Pathology, Department of Surgery, Johns Hopkins University and Hospital

The very low tissue toxicity of broth filtrates of penicillin culture was first demonstrated by Fleming (1929) for leucocytes, and is recorded in his original report on penicillin. He states, "*In vitro*, penicillin, which completely inhibits the growth of *Staphylococci* in a dilution of 1–600, does not interfere with leucocyte function to a greater extent than does ordinary broth." Fleming's nutrient broth evidently contained about twenty-five Oxford units per cubic centimeter of penicillin. Most of the work done on tissue toxicity since this early report has been carried out on purified concentrates. We have made some observations on both types of penicillin preparations, and some of our findings are presented in this communication. A summary of the effects of penicillin, as observed by different investigators on cells *in vitro*, is given in Table I. We believe that in many instances where fairly marked cytotoxicity was found, the toxicity was due more to the effects of substances added during the purification process than to any products of the mold.

In this laboratory, penicillin filtrates made by Dr. Fisher (1943), of the Department of Medicine, from penicillin culture were added to roller tube cultures of normal and malignant cell strains which were then studied for evidence of cytotoxicity. The simplified medium in which the penicillin was grown had little or no protein content and consisted only of the products of completely hydrolysed casein ("Casamino Acids"—Difco) in a concentration of three percent of the solid with two percent sugar added. This medium, according to Dr. Fisher, was relatively harmless to animals when administered in a number of ways. He made no critical tests for pyrogens as is done with the purified commercial preparations of penicillin. Also in this

[1] Presented at the A. A. A. S.—Gibson Island Conference on Cancer—July–August 1944.

preliminary work we did not test critically the cytotoxicity of this penicillin medium before the mold was grown in it. Some toxicity for tissue cultured cells was to be expected since to date we have no records of completely non-toxic commercially prepared protein hydrolysates or digests when any of these are added to continuous cultures at so-called physiological levels. In this connection, it is of interest to state that all of the amino acid mixtures, protein hydrolysates, and protein digests that were prepared for intravenous use and submitted to us for tests of cytotoxicity have been found to be quite toxic when added to continuous cultures.

In the experiments on crude penicillin filtrate, most of the tissue cultures used were taken from strains which had been under cultivation for a good many years, except one series of cultures of normal human lung. This group of cultures was explanted only eleven days before the penicillin filtrate treatment began. They showed good activity of epithelium and fibroblasts twelve days after continuous treatment with penicillin filtrate in a dilution of 1–30. The concentration was then stepped up to 1–10, and after sixty-nine days of exposure at this concentration they died out. It is our belief that this result could have been obtained with the filtrate without penicillin. As noted above, a control of this type should have been included in the preliminary work. From these observations and those made on other normal and malignant cell strains, which latter were exposed for periods up to two weeks, we concluded that the penicillin in the crude fitrate was not very cytotoxic nor did it manifest a greater deleterious action on malignant cells than on normal cells. No further work was done with crude filtrates because we desired to extend our work to highly purified penicillin as soon as suitable material could be made available. Sometime later, therefore, a series of experiments were carried out using Merck penicillin sodium. All of these experiments were run on some of the penicillin sodium which was contained in five vials of two lots, #172 and #196 of Merck and Co. The material was a pale whitish powder with an average potency of 932.8 Oxford units per mgm. The material in individual vials varied from 574 to 1500 units per milligram on the basis of the weights of material taken from vials labeled, "100,000 Oxford Units." Some of this therapeutic penicillin was therefore almost as potent as the crystalline penicillin

TABLE I

Summary of effects of penicillin as observed by different investigators on cells in vitro

	INVESTIGATORS	TISSUE AND CELL TYPE	MEDIUM	PERIOD OBSERVED	PENICILLIN PREPARATION	APPROXIMATE OXFORD UNITS PER MG. (cc.)	CONCENTRATION OF DRUG IN MEDIUM		RESULTS
							Dilution	Units/cc.	
1	Fleming (1929)	Human leucocytes	Broth	Not stated	Crude filtrate	25 (?)	Undiluted filtrate	25	No more toxic than broth
2	Chain, Florey, et al. (1940)	Human leucocytes	Ringer-Locke	3 hr.	Impure concentrate	Less than 5	1-1000	5	Leucocytes active for at least 3 hr.
3	Abraham, Florey, et al. (1941)	Human leucocytes	C. G. Paine's solution + 10% auto. serum	0–4 hr.	Purified "therapeutic penicillin" and more highly purified	45	1-100	450	Killed immediately
							1-250	180	50% survived 4 hr. but were sluggish
							1-500	90	Same activity as control medium
	" (& F. Jacoby)	Hen's blood macrophages	"	60+ hr.	"	"	1-200	225	Mitotic inhibition in 42 hr. with delayed recovery in fresh serum
							1-500	90	Mitosis reduced with quick recovery in fresh serum
							1-2000	22.5	Rapid mitosis. No after effects
							1-10,000	4.5	Rapid mitosis. No after effects

	"	10-day chick heart fibroblasts	"	48 hr. with drug followed by 3 to 4 passages in control media	"	"	1-800 1-1600 1-6000	56 28 7.5	Threshold concentration Minimal lethal concentration. Recovery on subculture possible after 48 hr. exposure Detectably toxic
	"	10-day chick epithelium (intestine, lung) late rat embryo kidney	"	"	"	"	1-1000 1-2000	45 22.5	Degenerate outgrowth and maceration Good proliferation on surface for 48 hr.
4	Herrell & Heilman (1943)	Emigrating cells of mesenteric lymph node of rabbit	1 pt.—chicken plasma; 3 pts.—auto. serum + chick embryo extract	24 hr.	Purified (Florey & Heatly). Not pyrogen-free	42	1-1000 1-2000 1-5000	42 21 8.4	Decrease in ameboid lymphocytes and degeneration of granulocytes. Erythrocytes unchanged No demonstrable effect on lymphocytic migration in 24 hr. No demonstrable effect on lymphocytic migration in 24 hr.
					Also Merck (1942)	28	1-500	56	Changes similar to 1-1000 (above) but with greater toxicity

TABLE I—*Concluded*

	INVESTIGATORS	TISSUE AND CELL TYPE	MEDIUM	PERIOD OBSERVED	PENICILLIN PREPARATION	APPROXIMATE OXFORD UNITS PER MG. (cc.)	CONCENTRATION OF DRUG IN MEDIUM		RESULTS
							Dilution	Units/cc.	
5	Rammelkamp & Keefer (1943)	Human leucocytes from cerebrospinal fluid after intrathecal injection	Cerebrospinal fluid	—	Squibb penicillin-sodium salt (yellow)	—	—	20 units equals highest level 6 hr. after penicillin injection	Leucocytes actively motile in samples of spinal fluid taken 6 to 96 hr. after 10,000 units given intrathecally
6	Gey (quoted by Fisher —1943)	Continuous cultures of human and rat normal and malignant cells	1 pt.—balanced salt solution; 1 pt.—bovine embryo extract; 4 pts. — human cord serum; 4 pts. — chicken plasma	Over 2 weeks	Fresh and stored (−32°C.) 2 weeks to 2 months) filtrates of penicillin culture	—	1-10 Undiluted filtrate	4.5 45	No toxic effects on tumor or normal cells after 2 weeks' continuous exposure 24-hr. exposure to full strength did not kill
7	Cornman (March 1944 & August 1944)	Primary explants of rat and mouse normal fibroblasts and sarcoma cells from 2 mouse and 6 rat tumors	Lewis' 61A medium (similar to Gey medium) and variations	For short periods	Squibb penicillin-sodium salt (yellow)	—	—	—	Proper dosage level kills all sarcoma cells without damaging normal fibroblasts. One mouse tumor showed *no* selective response

8	Shettles (1944)	Human spermatozoa	Seminal plasma diluted with normal saline	—	Penicillin - sodium salt	—	—	0.001 to 200,000	In dilutions well above those reached in tissue following therapy there was no impairment of grade of motility or percentage of motile cells as compared to control
9	Lewis (1944)	Primary explants of mouse sarcoma and normal muscle	Lewis' 61A medium (similar to Gey medium) and variations	For short periods	Merck penicillin-sodium salt Lot 193	—	—	120 to 160	No damage to sarcoma or normal cells in roller tube cultures
		Primary explants of mouse kidney, spleen, muscle & heart, and sarcoma	"	"	Merck colorless penicillin-sodium salt Lot 4R263	1500	—	20 to 160	Abundant growth 24 hr. after single treatment. Some got second treatment with 80 and 160 units and continued to grow for "a number of days"
		"	"	"	Squibb penicillin-sodium salt (yellow)	—	—	20 to 53 60 to 80 100 to 120	Abundant growth "Tumor cells damaged—normal cells growing" "Tumor cells killed—normal cells damaged"

sodium which is used as a standard and has a potency of 1650 Oxford Units. In the experiments described here, no yellow penicillin sodium was used.

METHOD

The procedures used were similar to those described by Gey and Gey (1936) for the maintenance of strains of cells by the roller tube method of Gey (1933). In this culture method, a plasma substrate made up of balanced salt solution 10 percent, bovine embryo extract 10 percent, human placental cord serum 40 percent, and chicken plasma 40 percent is added to standard 16 x 150 mm. pyrex test tubes in a quantity sufficient to coat two-thirds of the length of the tube with a thin film of medium. The plasma is always added first in order to insure good adhesion to the glass. Sodium heparin (Lederle), in a concentration of 1–250,000 or less in the plasma, is used to delay the clotting time to about a minute and thus provide adequate time to orient the tissue culture colonies in the tube. A supernatant fluid of the same composition as the substrate without the plasma is then added to the tubes. The ratio of supernatant to substrate is usually about 5–1.

The purified sodium penicillin was made up in suitable concentrations in the supernatant fluid before use. In the majority of the experiments, it was added continuously to the supernatant fluid with each bi-weekly replenishment of the medium. In some experiments it was added to both substrate and supernatant fluid. The importance of the manner in which the penicillin is added to the cultures will be discussed later. The penicillin powder and solutions, and all of the fluids derived from biological sources, were stored in a deep freeze box maintained at −40°C. In a few instances unfrozen chicken plasma was used.

EFFECTS OF PURIFIED PENICILLIN SODIUM

A large group of normal and malignant cell cultures previously maintained as pure strains in roller tubes were tested for toxicity of purified penicillin in the presence of a heavy growth of a nonhemolytic *Staphylococcus aureus* (Table III). In most instances, at the time of the initial treatment the microorganisms had almost completely overgrown the tissue culture colonies and had made the fluid quite turbid. Before the initial treatment with penicillin some of the cell

TABLE II

Summary of effects of sodium penicillin (Merck) on continuous cultures of Walker rat sarcoma 319[a] and rat thyroid mesenchyme strain M[b]

Treated in strictly parallel series, December 3, 1943

CONCENTRATION OF DRUG IN MEDIUM [c] (OXFORD UNITS)	CYTOLOGICAL RESPONSE AND SURVIVAL PERIOD	
	W.R.S. 319	Rat Thy. *M.*
No-penicillin control	Good growth. No evidence of toxicity	Good growth. No evidence of toxicity
Series treated with Sodium Penicillin:		
4000 units/cc.	Cytological changes in 3 hours. Killed in 1–2 days	Cytological changes in 3 hours. Killed in 4–6 days
2000 units/cc.	Cytological changes in 48 hrs. Killed in 3 weeks	Cytological changes in 72 hrs. Killed in 3 weeks
1000 units/cc.	Cytological changes in 72 hrs. Killed in 3 weeks	Cytological changes in 1 week. Dying in 4 weeks. *Recovers* on removal of sodium penicillin
200 units/cc.	Good growth. No evidence of toxicity	Good growth. No evidence of toxicity
100 units/cc.	Good growth. No evidence of toxicity	Good growth. No evidence of toxicity
20 units/cc. 10 units/cc. 2 units/cc. 1 units/cc. 0.2 units/cc. 0.1 units/cc.	Good growth. No evidence of toxicity	Good growth. No evidence of toxicity
pH	No evidence that results are correlated to pH pH range stayed within optimal physiological limits	No evidence that results are correlated to pH pH range stayed within optimal physiological limits

[a] Ten-year-old tissue cultured strain.

[b] Five-year-old tissue cultured strain.

[c] The control medium was made up of 2 parts of balanced salt solution (Gey) and 8 parts of a fluid medium containing: 1 part balanced salt solution, 1 part bovine embryo extract, and 4 parts of human placental cord serum. This combined fluid was used as supernatant and added to a thin film of substrate which included 40 percent chicken plasma. The penicillin was added to the 2 parts of balanced salt solution in the treated series.

cultures had been infected for a number of days but the cells were not damaged. Others included in the group outlined in Table III were

TABLE III

Summary of effects of penicillin sodium (Merck)[a] on pure strains of cells

Most treated initially in the presence of an abundant growth of a non-hemolytic *Staphylococcus aureus*

CODE NO. OF CELL STRAIN	SOURCE		APPROX. AGE OF STRAIN (MARCH 1944)	CONCENTRATION OF DRUG IN MEDIUM[b] (OXFORD UNITS PER CC.)	RANGE OF SURVIVAL PERIOD		
					Death after —— days	Damaged during —— days. Recovery possible with drug removed	Slight or no cytotoxic action. Indefinite cultivation possible
			years		*days*	*days*	
D-1 Re.....	Human	Chondromyxosarcoma (Altered)	10+	10,000		4 and 14	
A. Fi.....	Human	Fibrosarcoma	6	10,000	7 and 11		
A.R.F.-68.....	Rat	Normal cartilage	5.5	10,000	7 and 12		
Thy. M.....	Rat	Normal thyroid mesenchyme	5.5	10,000	7 and 15	3	
Rt. S1. G1.....	Rat	Normal salivary gland mesenchyme	5.5	10,000	14		
3G-29.....	Rat	Normal zona fasciculata of adrenal	5	10,000		7	
ANSAT-21[c]....	Rat	Altered normal subcutaneous areolar tissue	5.5	10,000	7	7 and 17	
TSAT-70 P2.....	Rat	Sarcoma from altered mesenchyme cultures	4+	10,000	7, 7, 10, 10, and 11		
TSAT-378-376...	Rat	Sarcoma from altered mesenchyme cultures	3	10,000	7	4, 7 and 7	
D-1 Re.....	Human	Chondromyxosarcoma	10+	5,000	11, 14, and	14 (?), 16, 26,	

D-1 Re.....	Human	Chondromyxosarcoma (Altered)	10+	5,000	11, 14, and 25	14 (?), 16, 26, 43, 71, 74, and 85[d]	
A.R.F.-68.....	Rat	Normal cartilage	5.5	5,000	94	43	
Thy. M.....	Rat	Normal thyroid mesenchyme	5.5	5,000		44 and 50	
Rt. S1 G1.....	Rat	Normal salivary gland mesenchyme	5.5	5,000		11	
Thy. 27A.....	Rat	Normal thyroid mesenchyme	5	5,000	36		
3G-29.....	Rat	Normal zona fasciculata of adrenal	5	5,000		22	
ANSAT-21.....	Rat	Altered normal subcutaneous areolar tissue	6	5,000		11, 15, 18 and 43	
TSAT-70 P2[o]...	Rat	Sarcoma from altered mesenchyme cultures	4+	5,000			Active growth 45 days—lost from other causes. Active growth 50 days—still growing
TSAT-72[o].....	Rat	Sarcoma from altered mesenchyme cultures	5.5	5,000	111 (?)		Active growth 69 days—still growing
TSAT-378-376...	Rat	Sarcoma from altered mesenchyme cultures	3	5,000			Active growth 69 and 78 days, then discontinued
D-1 Re.....	Human	Chondromyxosarcoma (Altered)	10+	1,000			Active growth 26, 107, and 107 days—lost from other causes
A. Fi.....	Human	Fibrosarcoma	6	1,000			Active growth 57 days—lost from other causes. Two series with active growth for 155 days and still growing

TABLE III—*Concluded*

CODE NO. OF CELL STRAIN	SOURCE		APPROX. AGE OF STRAIN (MARCH 1944)	CONCENTRATION OF DRUG IN MEDIUM [b] (OXFORD UNITS PER CC.)	RANGE OF SURVIVAL PERIOD		
					Death after —— days	Damaged during —— days. Recovery possible with drug removed	Slight or no cytotoxic action. Indefinite cultivation possible
			years		*days*	*days*	
WRS-319.....	Rat	Reticulum-cell sarcoma	10	1,000		3 series run 14	
NSAT-14pf.....	Rat	Normal subcutaneous areolar tissue	5.5	1,000		12 and 15	Active growth 57 days—lost from other causes
Rt. S1. G1.....	Rat	Normal salivary gland mesenchyme	5.5	1,000		26, 28 and 29	Active growth 89 days, then discontinued
3G-29.....	Rat	Normal zona fasciculata of adrenal	5	1,000			Fairly active growth 50 days, then drug removed
TSAT-70 P2.....	Rat	Sarcoma from altered mesenchyme cultures	4+	1,000			Active growth 50 days—still growing.[e] Active growth 31 days, then drug removed
TSAT-72.....	Rat	Sarcoma from altered mesenchyme cultures	5.5	1,000			Active growth 26 days, then drug removed
D-1 Re.....	Human	Chondromyxosarcoma (Altered)	10+	100			No evidence of toxicity. Good growth indefinitely[f]
A. Fi.....	Human	Fibrosarcoma	6	100			
WRS-319.....	Rat	Reticulum-cell sarcoma	10	100			
WRC-256.....	Rat	Carcinoma of the breast	3	100			
NSAT-14 pf.....	Rat	Normal subcutaneous areolar tissue	5.5	100			
ARF-68.....	Rat	Normal cartilage	5.5	100			
Thy. M.....	Rat	Normal thyroid mes-	5.5	100			

ARF-68.....	Rat	Normal cartilage	5.5	100			
Thy. M.....	Rat	Normal thyroid mesenchyme	5.5	100			
Rt. S1. G1.....	Rat	Normal salivary gland mesenchyme	5.5	100			
Thy. 27-A.....	Rat	Normal thyroid mesenchyme	5	100			
3G-29.....	Rat	Normal zona fasciculata of adrenal	5	100			
NSAT-21.....	Rat	Altered normal subcutaneous areolar tissue	6	100			
TSAT-70 P2....	Rat	Sarcoma from altered mesenchyme cultures	4+	100			No evidence of toxicity. Good growth indefinitely[f]
TSAT-72.....	Rat	Sarcoma from altered mesenchyme cultures	5.5	100			
TSAT-378–376..	Rat	Sarcoma from altered mesenchyme cultures	3	100			
BP-5.....	Rabbit	Epidermal carcinoma of scrotum	0.5	100			

(a) Lots #172 and #196 used.

(b) All of the strains listed were grown in stock media of the composition given under "Methods."

(c) This is one of the tumor-producing altered normal mesenchyme strains.

(d) The death in this group was not contributed primarily to action of penicillin.

(e) Treatment was begun on primary explants of rat tumor produced by penicillin-treated cultures and without the microorganism present.

(f) All of these cultures have been run for over six months with 100 Oxford units per cubic centimeter in several series, and have shown no evidence of cytotoxicity.

free of microorganisms. The survival of cells in continuous culture is possible under these conditions because most *Staphylococci* produce no noticeably toxic products capable of destroying the living cells. This is true, we have observed, only if the nutrient medium has not been exhausted by the microorganisms. The *Staphylococcus* toxin of Burky (1933) is perhaps an exception. Its cytotoxicity for cells ***in vitro*** has not been tested.

In a series run by Dr. Inui, the effects of a similar sodium penicillin (Merck) as that used in the above group were tested on Walker rat sarcoma 319 and rat thyroid mesenchyme strain *M*. In this group (Table II) great care was taken to run the cultures as a strictly parallel series. The composition of the medium differed only slightly from that used in the large group outlined in Table III. In the group run under strictly parallel conditions it will be noted that the records show that at 4000 Oxford units per cubic centimeter of medium the tumor cell strain (WRS 319) is killed in one to two days whereas the normal cell strain survives for a longer period of time, i.e., four to six days. As the dose is decreased a similar apparently differential toxicity is maintained down to a concentration of 1000 Oxford units per cubic centimeter of medium and at this level the tumor cells are killed in three weeks whereas the normal cells begin to die off at four weeks and can be recovered after the removal of the penicillin. These results should be interpreted with caution because the two types of cells involved do not have exactly the same cultural behavior. The tumor strain liquefies the medium fairly rapidly and by so doing the cells come into contact with the drug and its trace impurities always before the normal cells do. The normal cells, on the other hand, remain for many days in the clot before any of the colony outgrowth becomes exposed and thus are not in contact with the fluid for as much of the culture period as are the tumor cells.

In a culture medium cell exposure depends primarily upon such factors as clot diffusion, filtration, and the adsorption by the medium of any agents which affect cell activity. If, however, an agent such as penicillin does not penetrate the culture medium readily, it cannot be expected to influence greatly the activity of cells growing within that medium. In line with this, the excellent reports of Rammelkamp, Keefer and others (1943) indicate that penicillin penetrates poorly

through living tissue membranes. The variations in the survival periods and the variations in the dose tolerated by continuous cultures, we have observed, can in many instances be correlated with clot liquefaction. Thus in cultures in which the clot has liquefied the cells are directly exposed to the test fluid medium which, in this case, contains penicillin. In the work of Cornman (1944) and of Mrs. Lewis (1944), however, no statements are made with reference to the cultural behavior of the types of tumor and normal cells tested, especially regarding the ability of these cells to liquefy the medium. In their work only primary explants were used, whereas in most of the work reported here pure strains of cells maintained in continuous cultures were used.

As noted (Table III(o)) in a few instances, assays on purified penicillin were carried out on primary explants of rat tumors which were produced by inoculating cultures of tumor cell strains. In two series (TSAT-70 and TSAT-72) tumors were produced in rats by inoculating tissue cultured tumor strains which had been exposed for a number of days to 5000 units of purified penicillin, and when cultures were made of the tumors produced, they grew well when again exposed to 5000 units of purified penicillin. These recovered strains are still growing quite rapidly in continuous culture. Likewise, in the case of the altered human tumor strain D-1Re, now under cultivation for over ten years, the tumor cells even when exposed freely to the supernatant fluid of the culture medium tolerate fairly well a continuous level of 5000 units of penicillin. Tests of penicillinase activity (Abraham and Chain—1940) of these strains have not been made.

At higher concentrations, i.e., 10,000 units per cubic centimeter of medium, most cells in culture require special handling and at this level some cation correction for excess sodium is perhaps needed. The toxicity of various salts of penicillin has been correlated with the cation concentration by Welch and co-workers (1944). We have diluted cultures with distilled water to effect a correction for excess sodium, but the results are not convincing enough to report here. It is to be regretted that we have not run conclusive tests on yellow penicillin. Such tests would have permitted better comparison between the results tabulated here and those reported by Cornman and by Mrs. Lewis because a selective action on tumor cells was reported by them

for yellow preparations of penicillin. Further work on the various penicillin fractions is under way.

SUMMARY AND CONCLUSIONS

Continuous cultures of normal (rat) and tumor (human, rabbit, and rat) cells maintained in roller tubes as pure strains tolerate very high concentrations of penicillin sodium (Merck) for long periods of time.

The results indicate that it is possible to cultivate almost indefinitely strains of cells in plasma culture media whose supernatant fluid contains penicillin sodium in concentrations of 5000 Oxford units per cubic centimeter. Such successful cultivation apparently depends upon the character of the immediate cultural environment as well as the inherent tolerance of the cell strain.

No conclusive evidence of an increased tumor cell susceptibility to purified penicillin when compared to normal cells is justified from the results obtained on continuous cultures and on primary explants of tumors produced by inoculating continuous cultures of tumor cells into animals.

The toxicity of crude penicillin filtrate is much greater than that of the therapeutic penicillin sodium and is perhaps due in large part to the toxicity of the mold medium from which the filtrate is made.

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RAT BITE FEVER DUE TO STREPTOBACILLUS MONILIFORMIS TREATED WITH PENICILLIN

Case Report

ROBERT A. WEBER, M.D., and CUTTING B. FAVOUR, M.D.

From the Medical Clinic of the Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School, Boston

For centuries there has been recognized an intermittent febrile illness following the bite of a rat. In the Orient a form of rat bite fever called Sodoku (1) has been known since before the time of recorded medicine, and in this country Professor Eli Ives of Yale was lecturing on the subject (2) as early as 1831. The first etiological investigations, however, were not begun until 1900 when Miyake tried unsuccessfully to culture an organism from infected patients. Since that time there has been considerable controversy as to the causative agent (1, 3). At present it is apparent that two organisms cause symptoms subsequent to rat bite: Spirillum minus which produces the disease called Sodoku in the Orient; and Streptobacillus moniliformis (Haverhillia multiformis), the same organism that produced the well-studied, milk-borne epidemic of Haverhill fever (Erythema arthriticum epidemicum) which gave the organism one of its names (4).

Despite recent bacteriological advances, few cases have been reported where the causative organisms have been demonstrated by bacteriological methods. Because many of the earlier cases from which the clinical picture of the two forms of the disease is now drawn did not yield a Spirillum or a Streptobacillus in the laboratory, or, if they did, double infections were not ruled out, the differential between the two diseases remains uncertain. Brown and Nunmaker have pointed out (1) that there are many clinical variations in this disease. The diagnosis, therefore, should depend upon the isolation of the causative organism and the demonstration that it is the only infecting agent. Such accurate diagnosis is more than a matter of academic interest since the treatment and prognosis of the two forms of rat bite fever are quite different. Spirillum minus infections respond dramatically to arsenicals (1, 5). On the other hand, Strepto-

bacillus infections have reacted unpredictably to arsenicals, aurothiomalate and sulfonamides (1), cause months to years of severe disability and may terminate fatally (1). Because of this poor prognosis in Streptobacillus infections the present case of a recovery following penicillin therapy is presented.

CASE REPORT

B. S., a 25-year-old psychology instructor entered the Peter Bent Brigham Hospital on September 20, 1943, complaining of a rash and arthritis. Her past and family history were noncontributory.

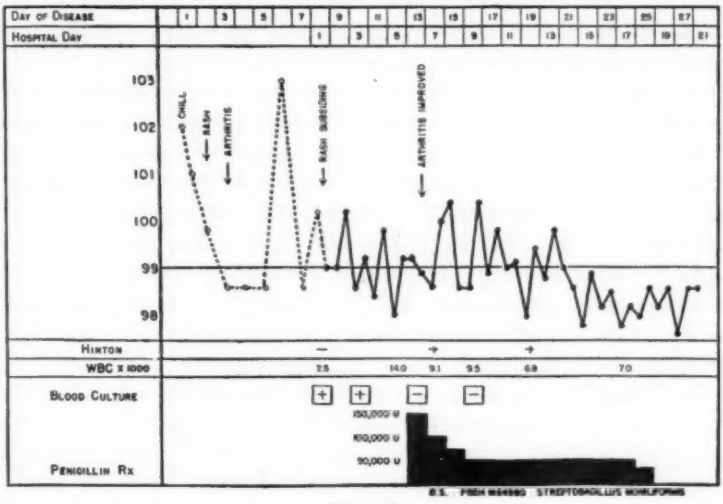

CHART 1

Nine nights before entering the hospital she was awakened by a shaking chill and generalized arthralgias (Chart 1). The following morning her temperature was 102°F. Salicylate therapy during the next two days did not relieve persistent arthralgias. The temperature, however, fell gradually to 99.8°. *Six days* before hospital admission red blotches appeared on the lateral aspects of her legs, on her face and later on the dorsa of her hands. These were believed to be due to aspirin, which was then discontinued. *Five days* before admission, her right wrist became sore, and *four days* before admission

she awoke with an arthritis of the left hip, knee and ankle which confined her to bed. Within two days symptoms had subsided sufficiently to permit the patient to attend an evening social function. The following morning, *two days* before admission, she again had generalized arthralgias and this time a temperature of 103°F. By the time of admission her temperature and symptoms had largely subsided. For eight days before admission she had been constipated and had a mild headache. For the five days before entry there was moderate nasal obstruction.

After hospitalization, it was discovered that on September 6, 1943, fourteen days before entry and five days before the abrupt onset of chills and fever, the patient had been bitten by a black laboratory rat in the course of a behavior training experiment.

Physical examination: The patient was a well-developed young woman who was lying comfortably in bed. The joints showed no deformity, redness or swelling, but were very tender to palpation and painful on motion. Over the lateral aspects of the lower extremities, on the face, and over the dorsa of the hands there were discrete erythematous 5 mm. macules with small, raised 1 mm. yellow necrotic centers. At the site of the rat bite there was a small, healed scar. The posterior pharyngeal wall was slightly injected. Physical examination was otherwise negative.

Laboratory data: Hemoglobin, white count and urine were not remarkable throughout the course in the hospital. An X-ray of the heart and lungs and an electrocardiogram were also normal. The blood Hinton test was negative on the ninth day of disease, positive on the fourteenth and twentieth days of disease and repeatedly negative after two months. Agglutination tests for Proteus Ox 19, Ox K, Ox 2 and Brucella were negative both on admission and three weeks later. Cultures of the nasopharynx and skin lesions were planted at the bedside on chocolate agar, Mueller's starch agar, and blood agar and immediately incubated in a candle jar. The nasopharyngeal culture grew out Neisseria catarrhalis, and the skin culture staphylococcus albus. Routine blood cultures consisting of 12 cc. of venous blood placed in 3 cc. of 2.5% sodium citrate and within 20 minutes planted as a blood agar pour plate and as blood broth (10 cc. of blood in 100 cc. of tryptic digest broth) were taken on the first,

third and subsequent hospital days (as indicated in Chart 1) and incubated in a candle jar. After forty-eight hours the diagnosis of Streptobacillus moniliformis was confirmed when characteristic (6, 7), small, fluffball colonies appeared in the blood broth. Stained smears of both positive cultures showed gram-negative, interwoven filaments and small, pleomorphic, gram-negative bacilli, both structures with bulb-like masses along their lengths. This organism retained its morphology through twenty-six subcultures.

To rule out a mixed infection with Spirillum minus, twelve white Swiss mice were injected immediately after venopuncture with the patient's whole blood 0.05 cc. intravenously and 0.2 cc. intraperitoneally. Culture of the same sample of blood grew out the Streptobacillus. On the fourth, twelfth, twenty-first, and thirty-second days, mouse bloods and peritoneal washings were examined under a darkfield microscope. No Spirilla were found in any of these specimens. Ordinarily mice used for the detection of Spirillum minus infections should have both peritoneal washings and blood examined before inoculation because of the frequency of naturally occurring Spirillum infections in laboratory mice (8).

Hospital course: On the eleventh day of the disease (fourth hospital day) at which time the blood culture became negative, the patient's rash began to subside and within seven more days had disappeared. At the same time the generalized arthritis noted on admission localized in the left shoulder and right elbow and became so painful that 10 mg. of morphine every four hours was necessary for relief. On the thirteenth day of her disease, while this arthritis was at its height and when the temperature had stabilized between 99° and 100°, penicillin 8000 units intramuscularly every two hours was started (100,000 units a day). On the third day of treatment the dose was changed to 6000 units every three hours (50,000 units a day) and continued for the next ten days. Blood cultures following treatment were negative. On the second day of penicillin treatment there was striking relief of the subjective symptoms and increased mobility of the affected joints. On the seventh day of treatment the temperature returned to normal where it remained thereafter. At the end of treatment the generalized arthralgias had disappeared, and the wrist had cleared completely. The left shoulder, however, was

still quite stiff and moderately painful on motion. An X-ray of the shoulder at this time was reported as normal. The patient was discharged on the twenty-first hospital day with moderate limitation of motion of the left shoulder and residual tenderness over the left subacromial bursa. One month later all signs and symptoms were gone. Until now, over a year later, there has been no recurrence of signs or symptoms of her disease.

Penicillin sensitivity of the Streptobacillus: Serial ten-fold dilutions of penicillin in 10 cc. of tryptic digest broth enriched with 10% horse serum were inoculated with 2 cc. of a 48-hour subculture of the Streptobacillus. Using a standard Staphylococcus as a control and correcting for the added dilution factor brought about by the large inoculum necessary to insure good growth in routine transfers, the organism was twice found to be inhibited by 0.001 units of penicillin per cc. Even though the patient's clinical course suggested that the organism was very sensitive to penicillin and that 0.001 units per cc. might be the correct sensitivity level, some months later the culture was revived from colonies kept growing on solid media and the organism's penicillin sensitivity rechecked. In numerous tests well-controlled with the same stock strain of Staphylococcus aureus of known unvarying sensitivity to penicillin, the Streptobacillus penicillin sensitivity was found to be 0.034 units per cc. It is interesting that the revived strain was much more difficult to subculture than the original; it grew less luxuriantly, and it too was not pathogenic for white Swiss mice. We do not know whether this change in sensitivity is a technical variation or is related to loss of virulence.

Under different cultural conditions the Streptobacillus moniliformis is known to occur as a variant in an extremely minute L_1 form which is capable of migrating through virus-passing filters (9, 10, 11). Using one of the early subcultures of this patient's Streptobacillus, Dienes (12) found that surface inocula grew as the bacillary form on media containing less than 0.001 in penicillin per cc. whereas the L_1 form grew on media containing up to 50 units per cc. This transfer to the L_1 form which penicillin seems to bring about and which the drug will not prevent is of clinical as well as bacteriologic interest. It obscures the mechanism of recovery as well as the mode of action of penicillin in vivo. The same phenomenon observed by Dienes on

solid media was studied in a different way by the writers. The tube of penicillin broth from one of the early penicillin resistance tests which contained a concentration of penicillin just sufficient to permit minimal growth and hence should contain L_1 as well as the Streptobacillus forms was seeded with a Staphylococcus aureus and divided in two portions. One portion was passed through a Seitz filter. On culture the filtrate contained only the Streptobacillus moniliformis indicating that the filter was impervious to bacteria of ordinary size and presumably passed only L_1 forms of the Streptobacillus. A culture of unfiltered material was overgrown with Staphylococci.

The original culture was passed through twenty-six subcultures in an effort to get a smooth form for agglutination studies. Unfortunately the strain was lost before it became smooth enough to use.

The Streptobacillus has been shown to be closely related to disease of the upper respiratory tract in rats (13) and, therefore, easily can be transmitted by biting. As was the case here, however, it may be difficult to recover the organisms by prosaic methods. Four black laboratory rats, one of which was believed to have bitten the patient, were obtained for study. The nasopharynx of each rat was irrigated with 1 cc. of tryptic digest broth. The washings from each rat were then injected intraperitoneally into three white Swiss mice (12 mice in all). None of these mice developed arthritis or became clinically ill.

The case herein reported of rat bite fever due to the Streptobacillus moniliformis and probably not to the Spirillum minus has several interesting aspects. Although the laboratory work seems to have excluded the possibility of a concurrent Spirillum minus infection, the fact that the Hinton test became positive during the illness and subsequently reverted to negative might suggest that there was a double infection. Too few cases have been reported where double infections have been ruled out to know whether the positive tests for syphilis which are common in Spirillum minus infections and uncommon in Streptobacillus moniliformis infections are not in reality all due to co-existing Spirillum infections. The clinical picture of the present patient belies the latter suggestion. It seems most likely that there was only one infection. The incubation period was five days; the initial lesions did not light up at the onset of fever; the onset was abrupt with a chill; there was an irregularly relapsing fever; the rash

appeared as the temperature came down; and there were two kinds of arhtritis, an early migrating arthritis corresponding to the period of septicemia, and later a more severe, localized arthralgia of the larger joints corresponding to the period of developing immunity. All of these things are in favor of a single infection in this patient.

In this case report it is not known that the use of penicillin is responsible for recovery. Altemeier, Snyder and Howe (15) have recently reported similar favorable outcomes following penicillin therapy in three patients with rat bite fever due to Streptobacillus moniliformis. Organisms cultured from the blood stream were also inhibited by penicillin in vivo. Despite the inherent inaccuracies of biological tests, especially on an organism requiring many special environmental conditions for survival, it would seem that the strain of Streptobacillus recovered from this patient was quite sensitive to penicillin and that the blood level expected with the dose used in treatment (14) was many fold the amount necessary to inhibit growth by in vitro standards. These findings are consistent with the prompt recovery the patient made from an infection which is usually protracted despite many forms of therapy.

SUMMARY

1. A case of rat bite fever due to the Streptobacillus moniliformis and probably not to the Spirillum minus is described.

2. The organism isolated from the patient's blood was found to be inhibited by as little as 0.001 units of penicillin per cc. in media which normally supported luxuriant growth.

3. Intramuscular penicillin therapy was associated with unusually prompt recovery. The patient has remained well up to the present time, over one year later.

The authors wish to acknowledge the valuable technical assistance of Miss Ethel Burnham, laboratory technician, for her work done in connection with this paper.

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